## AMENDMENTS

### In the Title

**Clean Copy of Amended Title**

Real-time Sequence Determination using a Tagged Polymerase and/or a Tagged Nucleotide Triphosphate

### In the Specification

**Clean Copy of Amended Page 6, lines 3-18**

A1

Conventional DNA sequencing strategies and methods are reliable, but time, labor, and cost intensive. To address these issues, some researchers are investigating fluorescence-based, single-molecule sequencing methods that use enzymatic degradation, followed by single-dNMP detection and identification. The DNA polymer containing fluorescently-labeled nucleotides is digested by an exonuclease, and the labeled nucleotides are detected and identified by flow cytometry (Davis *et al.,* 1991; Davis *et al.,* 1992; Goodwin *et al.,* 1997; Keller *et al.,* 1996; Sauer *et al.,* 1999; Werner *et al.,* 1999). This method requires that the DNA strand is synthesized to contain the fluorescently-labeled base(s). This requirement limits the length of sequence that can be determined, and increases the number of manipulations that must be performed before any sequence data is obtained. A related approach proposes to sequentially separate single (unlabeled) nucleotides from a strand of DNA, confine them in their original order in a solid matrix, and detect the spectroscopic emission of the separated nucleotides to reconstruct DNA sequence information (Ulmer, 1997; Mitsis and Kwagh, 1999; Dapprich, 1999). This is the approach that is being developed by Praelux, Inc., a company with a goal to develop single-molecule DNA sequencing. Theoretically, this latter method should not be as susceptible to length limitations as the former enzymatic degradation method, but it does require numerous manipulations before any sequence information can be obtained.

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The term monomer as used herein means any compound that can be incorporated into a growing molecular chain by a given polymerase. Such monomers include, without limitations, naturally occurring nucleotides (*e.g.*, ATP, GTP, TTP, UTP, CTP, dATP, dGTP, dTTP, dUTP, dCTP, synthetic analogs), precursors for each nucleotide, non-naturally occurring nucleotides and their precursors or any other molecule that can be incorporated into a growing polymer chain by a given polymerase. Additionally, amino acids (natural or synthetic) for protein or protein analog synthesis, mono saccharides for carbohydrate synthesis or other monomeric syntheses.

**Clean Copy of Amended Page 24, lines 6-9**

The present invention provides a composition comprising an exonuclease including at least one molecular and/or atomic tag located at or near, associated with or covalently bonded to a site on the agent, where a detectable property has a first value when the polymerase is in a first state and a second value when the polymerase is in a second state during monomer removal.

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**Clean Copy of Amended Page 24, lines 10-13**

The present invention provides a composition comprising an enzyme modified to produce a detectable response prior to, during and/or after interaction with an appropriately modified monomer, where the monomers are nucleotides, nucleotide analogs, amino acids, amino acid analogs, monosaccharides, monosaccharide analogs or mixtures or combinations thereof.

**Clean Copy of Amended Page 28, line 2**

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**Cooperatively Tagged Monomers and Tagged Polymerizing Agent**

**Clean Copy of Amended Page 28, lines 13-16**

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The present invention provides a composition comprising a cooperatively tagged polymerase and tagged monomers, where a detectable property of at least one of the tags changes when the tag are within a distance sufficient to cause a change in the intensity and/or frequency of emitted fluorescent light.

**Clean Copy of Amended Page 31, lines 17-18**

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Figure 4 depicts an image of a 20% denaturing polyacrylamide gel containing size separated radiolabeled products from DNA extension experiments involving γ-ANS-phosphate-dATP;

**Clean Copy of Amended Page 31, lines 22-25**

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Figure 6 depicts an image of (A) 6% denaturing polyacrylamide gel, (B) a lightened phosphorimage of the actual gel, and (C) an enhanced phosphorimage of the actual gel containing products generated in DNA extension reactions using γ-ANS-phosphate-dNTPs;

Figure 7 depicts an image of (A) the actual gel, (B) a lightened phosphorimage of the actual gel, and (C) an enhanced phosphorimage of the actual gel;

Figure 8 depicts data for the Klenow fragment from *E. coli* DNA polymerase I incorporation of gamma-modified nucleotides;

Figure 9 depicts data for the *Pfu* DNA polymerase incorporation of gamma-modified nucleotides;

Figure 10 depicts data for the HIV-1 reverse transcriptase incorporation of gamma-tagged nucleotides;

Figure 11 depicts experimental results for native T7 DNA polymerase and Sequenase

incorporation of gamma-tagged nucleotides; and

Figure 12 depicts reaction products produced when the four natural nucleotides (dATP, dCTP, dGTP and dTTP) are used in the synthesis reaction (solid line) and reaction products produced when base-modified nucleotides are used in the synthesis reaction.

## DETAILED DESCRIPTION OF THE INVENTION

**Clean Copy of Amended Page 32, lines 14-20**

The pattern of emission signals is collected, either directly, such as by an Intensified Charge Coupled Devise (ICCD) or through an intermediate or series of intermediates to amplify signal prior to electronic detection, where the signals are decoded and confidence values are assigned to each base to reveal the sequence complementary to that of the template. Thus, the present invention also provides techniques for amplifying the fluorescent light emitted from a fluorescent tag using physical light amplification techniques or molecular cascading agent to amplify the light produced by single-molecular fluorescent events.

**Clean Copy of Amended Page 35, lines 17-24**

In one embodiment of the single-molecule DNA sequencing system of this invention, a single tag is attached to an appropriate site on a polymerase and a unique tag is attached to each of the four nucleotides: dATP, dTTP, dCTP and dGTP. The tags on each dNTPs are designed to have a unique emission signature (*i.e.*, different emission frequency spectrum or color), which is directly detected upon incorporation. As a tagged dNTP is incorporated into a growing DNA polymer, a characteristic fluorescent signal or base emission signature is emitted due to the interaction of polymerase tag and the dNTP tag. The fluorescent signals, *i.e.*, the emission intensity and/or frequency, are then detected and analyzed to determine DNA base sequence.

**Clean Copy of Amended Page 37 line 11 to Page 38 line 29**

Suitable atomic tag for use in this invention include, without limitation, any atomic element amenable to attachment to a specific site in a polymerizing agent or dNTP, especially fluorescent dyes such as d-Rhodamine acceptor dyes including dichloro[R110], dichloro[R6G], dichloro[TAMRA], dichloro[ROX] or the like, fluorescein donor dye including fluorescein, 6-FAM, or the like; Acridine including Acridine orange, Acridine yellow, Proflavin, pH 7, or the like; Aromatic Hydrocarbon including 2-Methylbenzoxazole, Ethyl p-dimethylaminobenzoate, Phenol, Pyrrole, benzene, toluene, or the like; Arylmethine Dyes including Auramine O, Crystal violet, H2O, Crystal violet, glycerol, Malachite Green or the like; Coumarin dyes including 7-Methoxycoumarin-4-acetic acid, Coumarin 1, Coumarin 30, Coumarin 314, Coumarin 343,

Coumarin 6 or the like; Cyanine Dye including 1,1'-diethyl-2,2'-cyanine iodide, Cryptocyanine, Indocarbocyanine (C3)dye, Indodicarbocyanine (C5)dye, Indotricarbocyanine (C7)dye, Oxacarbocyanine (C3)dye, Oxadicarbocyanine (C5)dye, Oxatricarbocyanine (C7)dye, Pinacyanol iodide, Stains all, Thiacarbocyanine (C3)dye, ethanol, Thiacarbocyanine (C3)dye, n-propanol, Thiadicarbocyanine (C5)dye, Thiatricarbocyanine (C7)dye, or the like; Dipyrrin dyes including N,N'-Difluoroboryl-1,9-dimethyl-5-(4-iodophenyl)-dipyrrin, N,N'-Difluoroboryl-1,9-dimethyl-5-[(4-(2-trimethylsilylethynyl), N,N'-Difluoroboryl-1,9-dimethyl-5-phenydipyrrin, or the like; Merocyanines including 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), acetonitrile, 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaminostyryl)-4H-pyran (DCM), methanol, 4-Dimethylamino-4'-nitrostilbene, Merocyanine 540, or the like; Miscellaneous Dye including 4',6-Diamidino-2-phenylindole (DAPI), 4',6-Diamidino-2-phenylindole (DAPI), dimethylsulfoxide, 7-Benzylamino-4-nitrobenz-2-oxa-1,3-diazole, Dansyl glycine, H2O, Dansyl glycine, dioxane, Hoechst 33258, DMF, Hoechst 33258, H2O, Lucifer yellow CH, Piroxicam, Quinine sulfate, 0.05 M H2SO4, Quinine sulfate, 0.5 M H2SO4, Squarylium dye III, or the like; Oligophenylenes including 2,5-Diphenyloxazole (PPO), Biphenyl, POPOP, p-Quaterphenyl, p-Terphenyl, or the like; Oxazines including Cresyl violet perchlorate, Nile Blue, methanol, Nile Red, Nile blue, ethanol, Oxazine 1, Oxazine 170, or the like; Polycyclic Aromatic Hydrocarbons including 9,10-Bis(phenylethynyl)anthracene, 9,10-Diphenylanthracene, Anthracene, Naphthalene, Perylene, Pyrene, or the like; polyene/polyynes including 1,2-diphenylacetylene, 1,4-diphenylbutadiene, 1,4-diphenylbutadiyne, 1,6-Diphenylhexatriene, Beta-carotene, Stilbene, or the like; Redox-active Chromophores including Anthraquinone, Azobenzene, Benzoquinone, Ferrocene, Riboflavin, Tris(2,2'-bipyridyl)ruthenium(II), Tetrapyrrole, Bilirubin, Chlorophyll a, diethyl ether, Chlorophyll a, methanol, Chlorophyll b, Diprotonated-tetraphenylporphyrin, Hematin, Magnesium octaethylporphyrin, Magnesium octaethylporphyrin (MgOEP), Magnesium phthalocyanine (MgPc), PrOH, Magnesium phthalocyanine (MgPc), pyridine, Magnesium tetramesitylporphyrin (MgTMP), Magnesium tetraphenylporphyrin (MgTPP), Octaethylporphyrin, Phthalocyanine (Pc), Porphin, Tetra-t-butylazaporphine, Tetra-t-butylnaphthalocyanine, Tetrakis(2,6-dichlorophenyl)porphyrin, Tetrakis(o-aminophenyl)porphyrin, Tetramesitylporphyrin (TMP), Tetraphenylporphyrin (TPP), Vitamin B12, Zinc octaethylporphyrin (ZnOEP), Zinc phthalocyanine (ZnPc), pyridine, Zinc tetramesitylporphyrin (ZnTMP), Zinc tetramesitylporphyrin radical cation, Zinc tetraphenylporphyrin (ZnTPP), or the like; Xanthenes including Eosin Y, Fluorescein, basic ethanol, Fluorescein, ethanol, Rhodamine 123, Rhodamine 6G, Rhodamine B, Rose bengal, Sulforhodamine

101, or the like; or mixtures or combination thereof or synthetic derivatives thereof or FRET fluorophore-quencher pairs including DLO-FB1 (5'-FAM/3'-BHQ-1) DLO-TEB1 (5'-TET/3'-BHQ-1), DLO-JB1 (5'-JOE/3'-BHQ-1), DLO-HB1 (5'-HEX/3'-BHQ-1), DLO-C3B2 (5'-Cy3/3'-BHQ-2), DLO-TAB2 (5'-TAMRA/3'-BHQ-2), DLO-RB2 (5'-ROX/3'-BHQ-2), DLO-C5B3 (5'-Cy5/3'-BHQ-3), DLO-C55B3 (5'-Cy5.5/3'-BHQ-3), MBO-FB1 (5'-FAM/3'-BHQ-1), MBO-TEB1 (5'-TET/3'-BHQ-1), MBO-JB1 (5'-JOE/3'-BHQ-1), MBO-HB1 (5'-HEX/3'-BHQ-1), MBO-C3B2 (5'-Cy3/3'-BHQ-2), MBO-TAB2 (5'-TAMRA/3'-BHQ-2), MBO-RB2 (5'-ROX/3'-BHQ-2); MBO-C5B3 (5'-Cy5/3'-BHQ-3), MBO-C55B3 (5'-Cy5.5/3'-BHQ-3) or similar FRET pairs available from Biosearch Technologies, Inc. of Novato, CA, tags with nmr active groups, tags with spectral features that can be easily identified such as IR, far IR, visible UV, far UV or the like.

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**Clean Copy of Amended Page 46, line 13**

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**Amino Acid Site Selection for the *Taq* Polymerase**

**Clean Copy of Amended Page 54, lines 23-31**

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The present invention uses tagged dNTPs or ddNTPs in combination with polymerase for signal detection. The dNTPs are modified at phosphate positions (alpha, beta and/or gamma) and/or other positions of nucleotides through a covalent bond or affinity association. The tags are designed to be removed from the base before the next monomer is added to the sequence. One method for removing the tag is to place the tag on the gamma and/or beta phosphates. The tag is removed as pyrophosphate dissociates from the growing DNA sequence. Another method is to attach the tag to a position of on the monomer through a cleavable bond. The tag is then removed after incorporation and before the next monomer incorporation cleaving the cleavable bond using light, a chemical bond cleaving reagent in the polymerization medium, and/or heat.

**Clean Copy of Amended Page 55, lines 6-12**

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where FR is a fluorescent tag, L is a linker group, X is either H or a counterion depending on the pH of the reaction medium, Z is a group capable of reaction with the hydroxyl group of the pyrophosphate and Z' is group after reaction with the dNMP. Preferably, Z is Cl, Br, I, OH, SH, $NH_2$, NHR, $CO_2H$, $CO_2R$, SiOH, SiOR, GeOH, GeOR, or similar reactive functional groups, where R is an alkyl, aryl, aralkyl, alkaryl, halogenated analogs thereof or hetero atom analogs thereof and Z' is O, NH, NR, $CO_2$, SiO, GeO, where R is an alkyl, aryl, aralkyl, alkaryl, halogenated analogs thereof or hetero atom analogs thereof.

**Clean Copy of Amended Page 57 line27 to Page 58 line 2**

**TABLE V**

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Primer Strand:
     TOP   5' GGT ACT AAG CGG CCG CAT G 3'          (SEQ ID NO. 1)
Template Strands:
     BOT-T 3' CCA TGA TTC GCC GGC GTA CTC 5'        (SEQ ID NO. 2)
     BOT-C 3' CCA TGA TTC GCC GGC GTA CCC 5'        (SEQ ID NO. 3)
     BOT-G 3' CCA TGA TTC GCC GGC GTA CGC 5'        (SEQ ID NO. 4)
     BOT-A 3' CCA TGA TTC GCC GGC GTA CAC 5'        (SEQ ID NO. 5)
     BOT-3T 3' CCA TGA TTC GCC GGC GTA CTT TC 5'    (SEQ ID NO. 6)
     BOT-Sau 3' CCA TGA TTC GCC GGC GTA CCT AG 5'   (SEQ ID NO. 7)
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**Clean Copy of Amended Page 64, lines 1-10**

*Taq* Pol I forward

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5'-gc gaattc atgaggggga tgctgcccct ctttgagccc-3' (SEQ ID NO. 8)

*Taq* Pol I reverse

5'-gc gaattc accctccttgg cggagcgc cagtcctccc-3' (SEQ ID NO. 9)

The underlined segment of each synthetic DNA oligonucleotide represents engineered EcoRI restriction sites immediately preceding and following the *Taq* pol I gene. PCR amplification using the reverse primer described above and the following forward primer created an additional construct with an N-terminal deletion of the gene:

*Taq* Pol I_A293_trunk

5'-aatccatgggccctggaggaggc cccctggcccccgc-3' (SEQ ID NO. 10)

**Clean Copy of Amended Page 64, lines 16-21**

**Mutagenesis**

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Once a suitable construct is generated, individual cysteine mutations are introduced at preferred amino acid positions including positions 513-518, 643, 647, 649 and 653-661 of the native *Taq* polymerase having the following amino acid sequence:

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1   mrgmlplfep kgrvllvdgh hlayrtfhal kglttsrgep vqavygfaks llkalkedgd
61  avivvfdaka psfrheaygg ykagraptpe dfprqlalik elvdllglar levpgyeadd
121 vlaslakkae kegyevrilt adkdlyqlls drihvlhpeg ylitpawlwe kyglrpdqwa
181 dyraltgdes dnlpgvkgig ektarkllee wgsleallkn ldrlkpaire kilahmddlk
241 lswdlakvrt dlplevdfak rrepdrerlr aflerlefgs llhefglles pkaleeapwp
301 ppegafvgfv lsrkepmwad llalaaargg rvhrapepyk alrdlkearg llakdlsvla
361 lreglglppg ddpmllayll dpsnttpegv arryggewte eageraalse rlfanlwgrl
421 egeerllwly reverplsav lahmeatgvr ldvaylrals levaeeiarl eaevfrlagh
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481 pfnlnsrdql ervlfdelgl paigktektg krstsaavle alreahpive kilqyreltk
541 lkstyidplp dlihprtgrl htrfnqtata tgrlsssdpn lqnipvrtpl gqrirrafia
601 eegwllvald ysqielrvla hlsgdenlir vfqegrdiht etaswmfgvp reavdplmrr
661 aaktinfgvl ygmsahrlsq elaipyeeaq afieryfqsf pkvrawiekt leegrrrgyv
721 etlfgrrryv pdlearvksv reaaermafn mpvqgtaadl mklamvklfp rleemgarml
781 lqvhdelvle apkeraeava rlakevmegv yplavpleve vgigedwlsa ke (SEQ ID NO. 11).

The following amino acid residues correspond to the amino acids between amino acid 643 and 661, where xxx represents intervening amino acid residues in the native polymerase:

643-Ala xxx xxx xxx Phe xxx Val xxx xxx Glu Ala Val Asp Pro Leu Met Arg Arg Ala -661 (SEQ ID NO. 12).

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**Clean Copy of Amended Pages 65 line 1 to Page 67 line 1**

Alanine 643 to Cysteine Replacement

*Taq* Pol I_Ala643Cys_fwd

5'-C CAC ACG GAG ACC tgC AGC TGG ATG TTC GGC G-3' (SEQ ID NO. 13)

*Taq* Pol I_Ala643Cys_rev

5'-C GCC GAA CAT CCA CGA Gca GGT CTC CGT GTG G-3' (SEQ ID NO. 14)

Phenylalanine 647 to Cysteine Replacement

*Taq* Pol I_Phe647Cys_fwd

5'-CC GCC AGC TGG ATG TgC GGC GTC CCC CGG GAG GCC-3' (SEQ ID NO. 15)

*Taq* Pol I_Phe647Cys_rev

5'-GGC CTC CCG GGG GAC GCC GcA CAT CCA CGT GGC GG-3' (SEQ ID NO. 16)

Valine 649 to Cysteine Replacement

*Taq* Pol I_Val649Cys_fwd

5'-GCC AGC TGG ATG TTC GGC tgC CCC CGG GAG GCC GTG G-3' (SEQ ID NO. 17)

*Taq* Pol I_Val649Cys_rev

5'-C CAC GGC CTC CCG GGG Gca GCC GAA CAT CCA GCT GGC-3' (SEQ ID NO. 18)

Glutamic Acid 652 to Cysteine Replacement

*Taq* Pol I_Glu652Cys_fwd

5'-GGC GTC CCC CGG tgc GCC GTG GAC CCC CTG ATG CGC-3' (SEQ ID NO. 19)

*Taq* PolI_Glu652Cys_rev

5'-GCG CAT CAG GGG GTC CAC GGC gca CCG GGG GAC GCC-3' (SEQ ID NO. 20)

Alanine 653 to Cysteine Replacement

*Taq* Pol I_Ala653Cys_fwd

5'-GGC GTC CCC CGG GAG tgC GTG GAC CCC CTG ATG CGC-3' (SEQ ID NO. 21)

*Taq* Pol I_Ala653Cys_rev

5'-GCG CAT CAG GGG GTC CAC Gca CTC CCG GGG GAC GCC-3' (SEQ ID NO. 22)

Valine 654 to Cysteine Replacement

*Taq* Pol I_Val654Cys_fwd

5'-GTC CCC CGG GAG GCC tgt GAC CCC CTG ATG CGC-3' (SEQ ID NO. 23)

*Taq* PolI_Val654Cys_rev

5'-GCG CAT CAG GGG GTC aca GGC CTC CCG GGG GAC-3' (SEQ ID NO. 24)

Aspartic Acid 655 to Cysteine Replacement

*Taq* Pol I_D655C_fwd

5'-CCC CGG GAG GCC GTG tgC CCC CTG ATG CGC CGG-3' (SEQ ID NO. 25)

*Taq* Pol I_D655C_rev

5'-CCG GCG CAT CAG GGG Gca CAC GGC CTC CCG GGG-3' (SEQ ID NO. 26)

Proline 656 to Cysteine Replacement

*Taq* Pol I_Pro656Cys_fwd

5'-CGG GAG GCC GTG GAC tgC CTG ATG CGC CGG GCG-3' (SEQ ID NO. 27)

*Taq* Pol I_Pro656Cys_rev

5'-CGC CCG GCG CAT CAG Gca GTC CAC GGC CTC CCG-3' (SEQ ID NO. 28)

Leucine 657 to Cysteine Replacement

*Taq* Pol I_Leu657Cys_fwd

5'-GCC GTG GAC CCC tgc ATG CGC CGG GCG GCC-3' (SEQ ID NO. 29)

*Taq* Pol I_Leu657Cys_rev

5'-GGC CGC CCG GCG CAT gca GGG GTC CAC GGC-3' (SEQ ID NO. 30)

Methionine 658 to Cysteine Replacement

*Taq* Pol I_Met658Cys_fwd

5'-GCC GTG GAC CCC CTG tgt CGC CGG GCG GCC-3' (SEQ ID NO. 31)

*Taq* Pol I_Met658Cys_rev

5'-GGC CGC CCG GCG aca CAG GGG GTC CAC GGC-3' (SEQ ID NO. 32)

Arginine 659 to Cysteine Replacement

*Taq* Pol I_Arg659Cys_fwd

5'-GCC GTG GAC CCC CTG ATG tGC CGG GCG GCC AAG ACC-3' (SEQ ID NO. 33)

*Taq* Pol I_Arg659Cys_rev

5'-GGT CTT GGC CGC CCG GCa CAT CAG GGG GTC CAC GGC-3' (SEQ ID NO. 34)

Arginine 660 to Cysteine Replacement

*Taq* Pol I_Arg660Cys_fwd

5'-GAC CCC CTG ATG CGC tGc GCG GCC AAG ACC ATC-3' (SEQ ID NO. 35)

*Taq* Pol I_Arg660Cys_rev

5'-GAT GGT CTT GGC CGC gCa GCG CAT CAG GGG GTC-3' (SEQ ID NO. 36)

Alanine 661 to Cysteine Replacement

*Taq* Pol I_Ala661Cys_fwd

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5'-CCC CTG ATG CGC CGG tgc GCC AAG ACC ATC AAC-3' (SEQ ID NO. 37)

*Taq* Pol I_Ala661Cys_rev

5'-GTT GAT GGT CTT GGC gca CCG GCG CAT CAG GGG-3' (SEQ ID NO. 38)

**Clean Copy of Amended Page 68 line 20 to Page 69 line 7**

A18

In the first example, illustrates the incorporation of ANS-γ-phosphate dATP to produce extended DNA products from primer templates. The reactions were carried out in extension buffer and the resulting Radiolabeled products were size separated on a 20% denaturing polyacrylamide gel. Data was collected using a phosphorimaging system. Referring now the Figure 13, **Lane 1** contains 5' radiolabeled 'TOP' probe in extension buffer. **Lane 2** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 3** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 4** contains *Taq* DNA polymerase, 50 μM ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-Sau'). **Lane 5** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 6** contains spill-over from lane 5. **Lane 7** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 8** contains *Taq* DNA polymerase, 50 μM ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-T'). **Lane 9** contains *Taq* DNA polymerase, 50 μM dGTP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 10** contains *Taq* DNA polymerase, 50 μM dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 11** contains *Taq* DNA polymerase, ANS-γ-dATP incubated with a DNA duplex (radiolabeled TOP with excess 'BOT-3T'). **Lane 12** contains 5' radiolabeled 'TOP' probe in extension buffer. **Lane 13** contains 5'

radiolabeled 'TOP' probe and *Taq* DNA polymerase in extension buffer. Oligonucleotide sequences are shown in Table V.

**Clean Copy of Amended Page 69 line21 to Page 70 line 20**

This next example illustrates the synthesis of extended DNA polymers using all four ANS tagged γ-phosphate dNTPs. Products generated in these reactions were separated on a 20% denaturing polyacrylamide gel, the gel was dried and imaged following overnight exposure to a Fuji BAS1000 imaging plate. Referring now to Figure 14, an image of (A) the actual gel, (B) a lightened phosphorimage and (C)) an enhanced phosphorimage. Lane descriptions for A, B, and C follow: **Lane 1** is the control containing purified 10-base primer extended to 11 and 12 bases by template-mediated addition of alpha-$^{32}$P dCTP. **Lane 2** includes the same primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes (to denature template), the reaction was brought to 37°C (to anneal primer-template), *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 3** includes the same labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was DNA polymerase and all four gamma-modified dNTPs (100 μM, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 4** includes the control, purified 10-base primer that was extended to 11 and 12 bases by the addition of alpha-$^{32}$P-dCTP was cycled in parallel with lanes 5-8 reactions. **Lane 5** includes the same $^{32}$P-labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 6** includes the same $^{32}$P-labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μM, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 7** includes nonpurified, 10-base, $^{32}$P-labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 8** includes nonpurified, 10-base, $^{32}$P-labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs were added. The reaction was cycled

25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. Evident in the reactions involving tagged dNTPs is a substantial decrease in pyrophosphorolysis as compared to reactions involving natural nucleotides.

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**Clean Copy of Amended Page 70 line 21 to Page 71 line 21**

A20

This next example illustrates the synthesis of long DNA polymers using all four ANS tagged γ-phosphate dNTPs. Each primer extension reaction was split into two fractions, and one fraction was electrophoresed through a 20% denaturing gel (as described above), while the other was electrophoresed through a 6% denaturing gel to better estimate product lengths. The gel was dried and imaged (overnight) to a Fuji BAS1000 imaging plate. Referring now to Figure 15, an image of (A) the actual gel, (B) a lightened phosphorimage of the actual gel, and (C)) an enhanced phosphorimage of the actual gel. Lane descriptions for A, B, and C follow: **Lane 1** includes 123 Marker with size standards indicated at the left of each panel. **Lane 2** contains the control, purified 10-base primer extended to 11 and 12 bases by template-mediated addition of alpha-$^{32}$P dCTP. **Lane 3** contains the same $^{32}$P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes (to denature template), the reaction was brought to 37°C (to anneal primer-template), *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 4** includes the same $^{32}$P -labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C, *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μM, each) were added and the reaction was incubated at 37°C for 60 minutes. **Lane 5** includes the control, purified 10-base primer that was extended to 11 and 12 bases by the addition of alpha-$^{32}$P -dCTP was cycled in parallel with lanes 5-8 reactions. **Lane 6** includes the same $^{32}$P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 7** includes the same $^{32}$P -labeled primer that was incubated with double-stranded plasmid DNA at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four gamma-modified dNTPs (100 μM, each) were added. The reaction was cycled 25 times at 96°C for 10 seconds, 37°C for 1 minute, and 70°C for 5 minutes. **Lane 8** includes nonpurified, 10-base, $^{32}$P -labeled primer that was incubated with double-stranded DNA plasmid at 96°C for 3 minutes, the reaction was brought to 37°C for 10 minutes, during which time *Taq* DNA polymerase and all four natural dNTPs (100 μM, each) were added. The reaction was